CODON-OPTIMISED CRYIDA NUCLEIC ACID MOLECULE, NUCLEIC ACID CONSTRUCT, VECTOR, HOST CELL, PLANT CELL, TRANSGENIC PLANT, METHOD FOR TRANSFORMING A CELL, METHOD FOR PRODUCING A TRANSGENIC PLANT, METHOD FOR CONTROLLING INVERTEBRATE PESTS OF CROP PLANTS, AND USES OF THE NUCLEIC ACID MOLECULE

FIELD OF THE INVENTION

[0001] The present invention relates to new codon-optimized cry1 Da nucleic acid molecules from a gene sequence isolated from bacterium *Bacillus thuringiensis*. These molecules are used in the preparation of nucleic acid constructs, vectors and host cells, allowing the production of transgenic plants, such as corn, resistant to invertebrate pests, such as insects from the order Lepidoptera, particularly *Spodoptera frugiperda* (Noctuidae, Lepidoptera) and Diatrea *saccharalis* (Crambidae, Lepidoptera). Transgenic plant cells and plants comprising the molecules or constructs of the invention are also objects of the present invention. In particular, the transgenic plants according to the present invention are able to control caterpillars of the cited species that have become resistant to plants containing the cry1F gene. In addition, the present invention relates to a method for transforming a cell, a method of controlling invertebrate pests in crop plants and uses of nucleic acid molecules or constructs in the production of transgenic plants and for controlling invertebrate pests.

BACKGROUND OF THE INVENTION

[0002] Consistent advances in genetic engineering techniques have enabled the development of transgenic plants of commercial importance, containing the heterologous genes of interest, which can confer desirable traits to such plants. From among the genes of interest are genes that confer on plants resistance to herbicides, environmental stresses, and invertebrate pests, for example.

[0003] In the context of genes that code for proteins useful for controlling invertebrate pests, cry gene, derived from the Gram-positive bacterium *Bacillus thuringiensis* (Bt), can be mentioned. Said bacteria, which occurring naturally in several habitats, including the soil, phylloplane, grain residues, dust, water, plant matter and insects, has the innate characteristic of forming protein crystals during the stationary and/or sporulation phase. Protein crystals or delta-endotoxins, representing 20 to 30% of the total cell protein (Boucias & Pendland, 1998), have specific insecticidal properties and can be of various shapes, such as: bipyramidal, spherical, rectangular, cuboid and irregular. Bipyramidal crystals have a higher frequency of toxicity than crystals of other shapes, acting particularly against lepidopterans.

[0004] The mechanism of action of Cry proteins involves, in general, the solubilization of crystals in the midgut of insect larvae, the action of proteases on pro-toxins, adherence of active toxins to midgut receptors and the insertion of said active toxins into the apical cell membrane, creating ion channels or pores (cytolysis).

[0005] An advantage from the use of Cry proteins is the activity thereof against various insect species, being considered safe in relation to other organisms, such as mammals. Another advantage is the relative to the specificity thereof towards pest insects from different crops. Several cry genes are known. Cry1, cry2 and cry9 genes are generally active against lepidopterans; cry2, cry4A, cry10, cry11, cry17, cry19, cry24, cry25, cry27, cry29, cry30, cry32, cry39 and cry40 genes are generally active against dipterans; cry3, cry7 and cry8 genes are generally active against coleopterans; and cry5, cry12, cry13 and cry14 genes are generally active against nematodes.

[0006] Bt-based formulations available on the market represent a high percentage of sales of biopesticides and have been used for over 40 years for the control of pests from the orders Lepidoptera and Diptera. Production of the first transgenic plants containing cry genes did not present satisfactory results. In general, the expression levels of native genes were lower than those necessary to provide adequate protection against the target species in the field. Such a low concentration of Cry proteins was due to, among other factors, an incompatibility between codons of the gene donor species (Bt bacteria) and the gene recipient species (plants of interest).

[0007] It is known that different species use preferred codons, in particular for coding proteins, and these codon variations can negatively affect gene expression in the context of transgenics (Gustafsson, 2004). For example, in maize, codon AAG is used preferentially over codon AAA for amino acid lysine (Liu, 2009). Due to this unique characteristic of different groups of organisms, insertion of native *B. thuringiensis* cry genes into plants leads to low expression of the Cry protein of interest.

[0008] Moreover, bacterial genes have a low C+G content, in contrast to plant genes (Cambel & Gowri, 1990; Murray et al., 1989). A+T-rich bacterial nucleotide sequences can be recognized by plants as splice sites (Liu, 2009), polyadenylation signals (Joshi, 1987; Diehn et al., 1998) or elements of RNA destabilization, such as ATTTA (Ohme-Takagi et al., 1993). Therefore, to increase the expression of a Bt bacterium cry gene in the recipient organism, the gene must be "recoded", not only to adapt it to the preferred amino acid codons, but also to bring them closer to the G+C content of the recipient organism.

[0009] Genetic manipulation of cry genes can be promising to improve the efficiency and cost/benefit relationship of bioinsecticides and transgenic plants expressing these genes. Different Bt isolates can show a very wide range of toxic activity against the same target species, and one isolate can be very active against one species and virtually inactive against another (Jarret & Burges, 1982). Some combinations of Cry proteins have even shown synergistic toxicity towards lepidopterans. These authors reported that bioassays have shown synergism between Cry1Aa and Cry1Ac proteins, while the mixture of Cry1Aa and Cry1Ab exhibited antagonism towards the control of *Lymantria dispar.*

[0010] Considering the diversity of responses achieved by combining Cry insecticidal proteins and pest insects, as well as the importance of controlling such insects in crop plants, studies intended to better elucidate the decisive characteristics for obtaining satisfactory insecticidal effects as well as those aimed at the development of new transgenic plants resistant to insects are of major importance.

[0011] In previous studies, Bt isolates were tested against *Spodoptera frugiperda* or fall armyworm in vitro and molecular characterization of the most efficient ones was performed. Isolation of Bt strains was confirmed by means